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**Title:** Photocatalytic redox reactions at the surface of liposomes  
**Issue Date:** 2015-11-12
Chapter 8

Summary, Conclusions & Outlook
8.1 Summary

8.1.1 Introduction (Chapters 1 & 2)

In the near future, global energy resources in the form of fossil fuels will not be able to sustain the increase in demand caused by our energy-hungry society. Alternative ways for the production of energy in a sustainable way are the only option to supply energy in the future. Sustainable energy sources such as wind or water cannot provide enough power for the large energy demand. Therefore, the energy from the sun in the form of (visible) light should be utilized. Solar energy conversion has been a hot topic in the scientific community for decades. Presently, the efficient conversion of visible light to electricity has been achieved using various types of solar cells. However, the price of efficient solar cells is high, and the energy they generate during the day cannot be stored for use during the night because of the limited efficiency and high costs of large-scale batteries. The electricity storage problem is actually a discrepancy between energy demand and energy production. Therefore, alternative ways to convert and store the energy of the sun are needed.

One of the alternatives to solar panels is to utilize visible light in a way that mimics natural photosynthesis. In natural photosynthesis the energy of visible light is directly used to perform redox reactions, which leads to high-energy products (such as O₂ and sugars) from low energy compounds (H₂O and CO₂). The reaction sequence required for such conversions is very complex, and thus a simpler system would be preferred. For instance, the splitting of water into its constituents H₂ and O₂ has been thoroughly studied in homogeneous solution or at the surface of semiconductors. Thus far, the only working systems are based on semiconducting nanomaterials functionalized at their surface with catalysts for the production of H₂ or O₂.² In homogeneous solution, only the isolated half-reactions, i.e., either water oxidation⁴ or proton reduction⁵ have been reported using sacrificial oxidants or reductants, respectively.

The most chemically complex half-reaction of the two is water oxidation, and it is the focus of this thesis. The reaction requires the transfer of 4 protons and 4 electrons; thus, any molecule catalyzing this reaction must thus stabilize a minimum of three intermediates. Therefore, extensive research has been devoted to the development of molecular catalysts that can perform the water-oxidation reaction rapidly and without decomposing. As of now, many water-oxidation catalysts (WOCs) have been developed based on Mn, Ru, Ir, Fe, Co, Ni and Cu.⁴ The most active and stable catalysts are based on Ru and Ir. A standard method
for the screening of novel WOCs is to add large amounts of chemical oxidant (such as CeIV) to the mixture and then to monitor dioxygen evolution. In comparison to chemical water oxidation, very little research has been devoted to the development of a stable photocatalytic system employing these catalysts and photosensitizers under light irradiation.

As visible light is an energy source that induces the separation of one electron and one hole, the elementary reactions in the stepwise oxidation of H2O can never occur at one time. Therefore, the intermediates that are formed during catalysis will have to exist for extended periods of time compared to the photochemical elementary reactions. These intermediates, however, naturally possess a higher chemical potential than the starting materials, because their formation was triggered by the energy of a photon. Thus, in homogeneous solution, the intermediates will collide with each other randomly as they are freely moving around, and the charges that were separated utilizing the energy of the photon have a high chance to recombine.

Photocatalysis and water splitting thus require charge recombination to be avoided. In nature such a problem is overcome by sequential unidirectional electron transfer. In the thylakoid membrane of chloroplasts the photosystems I and II each perform electron-transfer steps leading to the reduction of NADP⁺ and

![Figure 8.1. Schematic overview of the photocatalytic reactions discussed in this thesis. In the research described in Chapter 3 and 4, a photosensitizer (PS) was bound to the surface of a liposome and a photocatalytic reaction between electron donor (ED) and electron acceptor (EA) both dissolved in the surrounding aqueous solution was studied. In Chapter 5, the membrane of the liposome was used to segregate the ED dissolved in the inner water pool and the EA dissolved in the surrounding aqueous phase. An electron relay (ER) was required to bring the electrons outside the liposome. In Chapter 7, the ED was replaced by a water-oxidation catalyst (cat), and water oxidation was studied at the surface of the liposome.](image)
the oxidation of water, respectively. Between these two photosystems, a quinone-based electron relay transfers the electrons from photosystem II to I. In a biomimetic approach, we have investigated photocatalytic redox reactions occurring at the surface of liposomes, which form the simplest artificial model of natural lipid membranes. The different types of systems studied in this thesis are schematically depicted in Figure 8.1, and explained in detail below.

8.1.2 Photocatalytic reduction at the surface of charged lipid bilayers (Chapter 3 & 4)

In order to progress towards directional electron transfer using liposomes, we first studied the effect of charged liposomes on simple photocatalytic reactions in which one or two electrons are transferred to form a colored photoproduct. In these reactions a sacrificial electron donor (ED) was used in order to complete the catalytic cycle (Figure 8.1, left). Two photoinduced electron-transfer reactions were studied (Scheme 8.1): the photoreduction of methyl viologen (MV$^{2+}$) by cysteine (CysSH), and that of 5,5'-dithiobis(2-nitrobenzoic acid) (H$_2$DTNB) by triethanolamine (TEOA). Negatively or positively charged watersoluble zinc porphyrins were employed as photosensitizers, respectively, which readily adsorbed to the surface of the charged liposomes via electrostatic interactions. In such a design, the charge of the liposome was indeed chosen opposite to that of the porphyrin, i.e., of the same sign as the electron acceptor. In such conditions it was shown that the mere addition of the liposomes caused a large increase in the quantum yield of the formation of the reduced photoproduct, which was caused mainly by the prevention of static quenching of the porphyrin photosensitizer by the electron acceptor, i.e., by disruption of the

![Scheme 8.1. The electron acceptors used in Chapters 3 to 5 and their 1- or 2-electron reduction.](image-url)
PS:EA ground state complex. Due to their larger size, charged liposomes outcompete the electron acceptor by having stronger electrostatic interaction with the photosensitizer, which has a very positive outcome on the photocatalytic reaction.

In Chapter 4 we describe the detailed kinetics and mechanism of the photocatalytic reactions reported in Chapter 3, both in absence and in presence of the liposomes. In particular, this study demonstrates that the presence of the charged liposomes leads to a large decrease of the rate of oxidative quenching of the excited photosensitizer by the electron acceptor. This effect can be understood as the charge of the liposome is of the same sign as that of the electron acceptor, which prevents EA to reach the liposome surface where the excited photosensitizer has been formed. This decrease in the rate of oxidative quenching causes the overall quantum yield for the formation of MV$^{2+}$ to be substantially lower at a low concentration of MV$^{2+}$, i.e., at a concentration where static quenching is negligible in homogeneous conditions. At high concentrations of MV$^{2+}$, the conclusions of Chapter 3 are still valid of course. For the photocatalytic reduction of DTNB$^2^-$, the reduction of the rate of oxidative quenching is also observed and particularly strong. It even causes a shift in the overall mechanism of the reaction, from oxidative quenching of the porphyrin in homogeneous solution, to reductive quenching in the presence of liposomes. Thus, charged liposomes are shown to have a large effect on the rates of elementary reactions in photocatalysis, and especially on the rate of oxidative quenching, as the quencher is kept away from the membrane by electrostatic repulsion.

8.1.3 Transfer of electrons across a lipid bilayer membrane (Chapter 5)

The two systems discussed in Chapters 3 and 4 only utilize one side of the membrane of the liposome. As in nature electrons are transferred across the bilayer membrane in order to decrease the rates of charge recombination, we studied a system where membrane-insoluble electron acceptor and donor are kept separate on both sides of a liposome membrane. This system was inspired by a cell-counting assay called WST-1. In this assay, bioelectrons produced by a living cell are transferred from NADH inside the cell, to a tetrazolium electron acceptor, WST1$^-$ located outside the cell (see Figure 8.1, middle). In the WST-1 assay the membrane-soluble electron relay 1-methoxy-N-methylphenazinium cation (MMP$^+$, see Scheme 8.1) needs to be added for the electrons to be transported across the membrane, as both NADH and WST1$^-$ cannot cross biological membranes. In our experiments, instead of using NADH we
encapsulated the electron donor HEDTA$^{3-}$ inside the inner water pool of a neutral liposome, and anchored a porphyrin-based sensitizer to the bilayer using an alkyl tail (see Figure 8.2). Upon visible light excitation of the photosensitizers located on the inner monolayer of the membrane, electrons were transferred from the electron donor to MMP$^+$, which, like in the WST-1 assay, transported them from inside the liposome to the WST1$^-$ acceptor present outside the liposome. The electron transfer does not occur in the absence of MMP$^+$. In addition, the electron transfer occurs three times faster across the membrane than when HEDTA$^{3-}$ and the tetrazolium salt are both located at the same side (i.e., outside) of the liposome, showing that separation of reagents successfully reduces charge recombination. Lastly, direct reduction of the tetrazolium salt can occur in the presence of dioxygen as well, which is an important prerequisite since ultimately the sacrificial electron donor HEDTA$^{3-}$ should be replaced by a water-oxidation catalyst that produces dioxygen from H$_2$O. However, the reduced electron relay MMHP was found to be unstable in the presence of O$_2$. The electron transfer across the membrane can thus only occur in the absence of O$_2$, which would prevent using such electron relay for a future solar fuel production scheme, and highlights the critical role played by molecular electron relays in photocatalysis.

Figure 8.2. Schematic representation of the mechanism of electron transfer across a lipid bilayer membrane as shown in Chapter 5.

8.1.4 **Photocatalytic water oxidation: detailed mechanism in absence and in presence of liposomes (Chapter 6 & 7)**

The reduction of electron acceptors such as MV$^{2+}$, H$_2$DTNB or WST1$^-$ occurs readily at the surface of liposomes, even though the kinetics of excited state quenching are significantly decreased. However, these photoreductions are still driven by the irreversible oxidation of sacrificial electron donors such as CysSH,
TEOA, or HEDTA$^{3-}$, which cannot lead to a sustainable solar fuel production device. In Chapter 6 and 7 these donors were replaced with a water-oxidation catalyst that should extract the necessary electron directly from water, and produce O$_2$ as a “waste”.

Despite the impressive amount of work made on photocatalytic water oxidation the detailed kinetics of this type of systems is very complicated and it has been poorly investigated to date, even in homogeneous solution. We thus performed a detailed kinetic analysis of three photocatalytic systems made of [Ru(bpy)$_3$]$^{2+}$ as photosensitizer, Na$_2$S$_2$O$_8$ as sacrificial electron acceptor, and three known water-oxidation catalysts (WOCs) based on Ru, Co, and Ir. In the literature photocatalytic systems are usually characterized by reporting turnover frequencies (TOF) and turnover numbers (TON) calculated, by analogy with “dark” catalysis, from the amount of WOC introduced in the reaction. However, we show in Chapter 6 that the catalytic reaction occurring at the WOC is not limiting the overall rate of O$_2$ formation, and that the WOC itself is not the species that decomposes the fastest during photocatalysis. Instead, electron transfer from the WOC to the photosensitizer is the rate-determining step (Figure 8.3, right), and because of the instability of the photosensitizer-derived catalytic intermediate [Ru(bpy)$_3$]$^{3+}$ in neutral aqueous solution, it is the photosensitizer that decomposes first and limits the overall stability of the photocatalytic system. Therefore, we propose that the characterization of the performance of a

Figure 8.3. Schematic overview of photocatalytic water oxidation at the surface of liposomes (left) or in homogeneous solution (right). The fast and slow electron transfer steps are indicated.
photocatalytic system always take into account the species that is the least stable, and that the TON should be calculated from this most unstable species. Thus, we introduce PTON, the TON calculated from the amount of photosensitizer introduced in solution, and show how it evolves with different experimental parameters of our experiments. In general, our study also shows that optimization of photocatalytic water oxidation is more elaborate than the mere optimization of the water-oxidation catalyst, and that more efforts should be directed at developing more stable photosensitizers and optimizing overall photocatalytic systems rather than individual steps.

In Chapter 7, we describe the functionalization of the photosensitizer and of three WOCs with alkyl tails, and the preparation of 4-component liposomes, to study photocatalytic water oxidation at the surface of the lipid bilayer. According to our results, the first effect of anchoring of the photosensitizer and WOC to the membrane is the stabilization of the photocatalytic system. The low volume of the bilayer induces a concentration effect that decreases the spatial distance between PS and WOC. Such concentration tremendously enhances the rate of electron transfer between the WOC and the photosensitizer, thus preventing photosensitizer decomposition and increasing PTON. However, similar to what was observed in Chapter 3, 4, and 5, the rate of oxidative quenching of the photosensitizer by the electron acceptor is decreased substantially at the surface of the liposome, which decreases the overall photocatalytic rate (TOF) in comparison to homogeneous conditions. At the surface of liposomes it is thus the oxidative quenching reaction that limits the rate of O₂ production, instead of the electron transfer step from WOC to PS that is the rate-limiting step in homogeneous solution (Figure 8.3 left). In other words, performing a photocatalytic reaction at the surface of liposomes changes its mechanism and rate-determining step, compared to homogeneous conditions. In our case, the concentration of the unstable oxidized photosensitizer is lower with liposomes, which limits the decomposition of this species and leads to increased amounts of dioxygen at the cost of production rate.

8.2 General conclusions

To realize the production of fuels from sunlight and water, efficient photochemical charge separation must be combined with efficient water-oxidation catalysis. The latter of these two has advanced to a state where the catalyst turns over rapidly and with little decomposition. However, a single system that produces fuels and O₂ from a unique homogeneous solution cannot
exist. We therefore used liposomes as a supramolecular scaffold at which photocatalytic redox reactions are performed. In general, the supramolecular binding of a photosensitizer to a bilayer membrane will lead to a decrease in the rate of excited state quenching by electron donors or acceptors dissolved in the bulk aqueous solution, which leads to lower photo-induced electron transfer rates. However, several other effects that are more positive towards the outcome of the photochemical reaction, were also observed. The first positive effect is that also the rate of charge recombination (e.g., inhibition by the product) was decreased, for example for the photoreduction of MV$^{2+}$ or DTNB$^2$- (Chapter 3 & 4). In photocatalysis, the ratio of the rates of charge separation vs. that of charge recombination must be maximized. The concomitant lowering of both rates might still lead to a positive outcome if charge recombination is lowered more than charge separation. A second positive effect of liposomes is that phase separation of electron donors and acceptors by the membrane can lead to an increased photoelectro- transfer quantum yield (Chapter 5). This effect is also likely a consequence of reduced product inhibition, as the photoproduct is formed far from the photocatalytic site. Lastly, by anchoring multiple photoactive components such as a photosensitizer and a water-oxidation catalyst at the surface of liposomes, the rate of electron transfer from the catalyst to the photosensitizer can increase significantly as a result of higher local concentrations (Chapter 7). Liposomes thus represent a powerful supramolecular scaffold for tuning photocatalytic reactions. They also form a bridge between homogeneous solutions, where elaborate mechanistic studies can be performed but controlled electron transfer cannot be achieved, and semiconductor (nano)systems, which can control electron transfer rather well and perform the full water-splitting reaction, but at the cost of catalytic rates and of molecular understanding of the photocatalytic mechanism.

### 8.3 Outlook & Perspectives

The complete inhibition of charge recombination by phase separation of the electron donor and acceptor has not been realized in this thesis. Future work should address systems deprived of sacrificial agents in order to realize water splitting. In order to create such a water splitting system, an electron (and proton) relay is required that can be reversibly oxidized/reduced in the presence of the products H$_2$ and O$_2$, and can be physically separated after the initial photoreaction from the sites of photocatalysis in order to prevent recombination reactions. Liposomes provide a versatile scaffold to build a system that may
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separately perform the oxidation of water and the reduction of protons.\(^6\)\(^-\)\(^8\) A straightforward method to do so would be to use the lipid bilayer of the liposome as a boundary over which the electrons can be transferred using membrane-soluble electron relays (see Figure 8.4). Although the products \(O_2\) and \(H_2\) would be produced at physically separated sites (i.e., at different sides of the membrane), the two gasses could not be separated macroscopically, thus producing a dangerous (explosive) gas mixture that would be too expensive to separate.

![Figure 8.4. Single liposome artificial photosynthesis where catalysts for water oxidation (outside bilayer leaflet) and proton reduction (inner bilayer leaflet) are coupled photocatalytically by a membrane-soluble electron relay.](image)

A possible method of solving this problem would be to segregate macroscopically the sites of water oxidation and proton reduction, by positioning them on different liposomes (see Figure 8.5). Due to their large size liposomes can be hold by nano-filtration membranes whereas small molecules are allowed to pass through (see Chapter 3). Furthermore, to transport electrons from the water-oxidation site to the proton-reduction site, a water-soluble electron relay would be required that is stable in the presence of dioxygen. By utilizing the directional transfer of electrons through the liposome bilayer, charge recombination with the reduced electron relay could be prevented. In this way, both the protons and electrons travel through the solution from water-oxidation
site to proton-reduction site, whereas the products (H₂ and O₂) can escape the solution at macroscopically different sites.

![Flow system using the nano-scale dimensions of liposomes to separate the products of artificial photosynthesis, H₂ and O₂. An electron relay (ER) is required to transfer the electrons from the site of water oxidation (right compartment) to the site of proton reduction (left compartment).](image)

Nowadays however, most application-driven research towards water splitting focuses on semi-conductor (nano)structures, which have already overcome the problems of charge-recombination by effective charge separation at the semi-conductor/solution interface, and separate products due to the macroscopic segregation of water-oxidation and proton-reduction sites.²⁹ The study of photocatalysis on liposomes is more interesting from a fundamental point of view than for applications purposes, as it provides interesting insights on the mechanisms of photochemical reactions occurring at biological membranes. Similar mechanistic studies are difficult to perform on solid surfaces due to the more limited number of analytical methods available. However, considering the mechanistic changes observed in this work upon realizing photocatalysis at the surface of liposomes, we can expect significant mechanistic aspects of photocatalysis to be different when molecular photosensitizers and *e.g.* water oxidation or proton reduction catalysts are attached at the surface of truly heterogeneous semi-conductor (nano)systems.

### 8.4 References

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